THE INFLUENCE OF THE MICROFLORA ON THE ACCUMULATION OF IONS BY PLANTS

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INTRODUCTION

Plants growing in soil are surrounded by very large numbers of micro-organisms. Bacterial numbers of up to 10^{10}/g of soil have been recorded, although on average, in most soils there are likely to be about 2×10^9 bacteria/g (Clark 1967). However, with the exception of the nitrogen fixing organisms and ectotrophic mycorrhiza and to a lesser extent endotrophic mycorrhiza the possible effects of this very extensive microflora have been very largely ignored by plant physiologists concerned with the accumulation of inorganic nutrients by plants. On the other hand, soil microbiologists interested in the micro-organisms rather than in the plant have amassed much data on the activities of the flora. They have shown that the numbers and types of bacteria and fungi in the soil fluctuate greatly depending upon the proximity to roots, depths, moisture content, nutrient status, plant species and many other factors. For example Berezova (1965) has shown that in the rhizosphere of potatoes there are over sixty million bacteria/g of soil capable of breaking down calcium triphosphate and twenty million able to break down organic phosphate, while these numbers fall to two and one million respectively in soil a centimetre or so away from the roots. Other types of organisms showed similar differences in distribution. These findings encourage speculation as to the significance of the microflora in the inorganic nutrition of plants. A comparative study of the nutritional relationships of plants grown in the presence and absence of micro-organisms at the ambient laboratory level has therefore been undertaken. The first phase of this work concerned with plants growing in water culture is here described.

The choice of water culture for the initial investigation was determined by two factors; not only is it a much simpler system but the majority of current concepts regarding the accumulation of inorganic nutrients by plants are derived from such studies rather than from plants growing in soil.

For a number of reasons the phosphate ion was chosen for investigation; firstly, it occurs in the soil solution in very low concentrations, secondly, micro-organisms have a great avidity for it and thirdly, results of experiments on the uptake and distribution of phosphate from dilute solutions (Russell and Martin 1953; Loughman and Russell 1957) under clean but not sterile conditions appeared compatible with a marked effect of micro-organisms.

METHODS

In all experiments the uptake and distribution of phosphate in barley plants (*Hordeum vulgare* var. Maris Badger) grown in the absence of micro-organisms has been compared with that of plants under non-sterile conditions. The experimental procedure is described in detail elsewhere (Barber 1967a) but the salient facts are as follows.

The seeds are sterilized and thoroughly washed in sterile water and after germination the seedlings are individually screened for the presence of micro-organisms by growth for 4 days on nutrient agar. Obviously clean seedlings are then transferred to totally enclosed culture vessels and are either grown in the complete absence of micro-organisms by the use of sterilized apparatus, air and culture solutions or allowed to become infected with indigenous laboratory organisms by omitting these precautions.

The uptake and distribution of ^{32}P-labelled phosphate was determined from solutions of potassium phosphate of varying concentrations and pH (Barber and Loughman 1967; Barber 1967b). The effect of the carbohydrate status of the plant on the extent of microbial activity was determined by comparing the absorption of phosphate by plants previously kept for twenty-four hours in light or in darkness. Before plants were harvested and assayed for ^{32}P, samples of solution were withdrawn from the sterile cultures and plated on malt and nutrient agar; contaminated replicates were discarded.

RESULTS

1. *Effects of external concentration*

Table 1 shows the effects of micro-organisms on the uptake of phosphate in 1 hour from solutions of increasing concentration. Except at the lowest concentration examined (0·001 ppm P) micro-organisms had little effect

TABLE I

Effect of micro-organisms on the uptake and distribution of ^{32}P-labelled phosphate in young barley plants after absorption for one hour in solutions of potassium phosphate of different concentrations

		Solution concentration ppmP				
		0·001	0·01	0·1	1·0	10·0
Total uptake μg P	Sterile	0·302	1·39	8·00	42·9	78·7
	N. Sterile	0·091	2·24	8·22	48·0	84·9
% in shoot	Sterile	0·7	3·9	22·8	27·9	29·7
	N. Sterile	0·05	2·0	9·0	17·7	20·0
Distribution in roots (as % total root P):						
Soluble esters + inorganic P	Sterile	85·0	86·4	91·9	91·5	89·3
	N. Sterile	33·3	31·2	73·6	78·9	86·7
Nucleic acids	Sterile	7·8	6·5	3·2	5·8	6·0
	N. Sterile	49·3	41·3	16·2	16·9	9·5
Phospholipids + phosphoproteins	Sterile	7·1	7·0	4·7	2·7	4·6
	N. Sterile	17·3	27·4	10·2	3·9	3·4

on the total amount of phosphate taken up but the distribution of the absorbed ion was greatly affected. When micro-organisms were present, a considerably larger quantity of the absorbed phosphate was incorporated into nucleic acids, phospholipids and phosphoproteins in the roots and less was transferred to the shoots. These results would be expected if micro-organisms in and on the roots absorbed phosphate at the expense of the root cells thus reducing the amount available for transport to the shoot. Because of the very short generation time of the micro-organisms they would rapidly incorporate phosphate into complex organic compounds and since it is not possible to separate the roots from the attendant microflora (Jenny and Grossenbacher 1963; Parkinson 1965; Scott 1966) analyses of roots grown under non-sterile conditions will reflect the dual nature of the material. It would be expected that such effects should be reduced when the external concentration of phosphate is high as the requirements of both plants and micro-organisms would then be satisfied.

Some visual evidence of the effects of bacterial infection are provided by autoradiographs which show that under non-sterile conditions there

are localized sites of accumulation of ^{32}P-labelled phosphate around the periphery of transverse sections of roots (Barber, Sanderson and Russell 1968).

Beyond this, evidence that micro-organisms in the culture solution may reduce the effective ionic concentrations is provided by the fact that at the end of the experiment more phosphate can be stripped off the walls of the vessels which contained the non-sterile plants. In the subsequent experiments, some factors other than concentration which influence the extent of the microbial effects are examined.

2. *Effects of pH*

Many bacteria are very sensitive to hydrion concentration, their metabolic rate being much reduced at low pH. Since in water culture studies bacteria tend to predominate over fungi it has been shown, not surprisingly, that the magnitude of the effects of micro-organisms demonstrated above is considerably influenced by pH (Barber 1967b). These effects are quite distinct from those caused by the change in the valency of the dominant phosphate ion in solution from the monovalent $H_2PO_4^-$ at low pH to the divalent HPO_4^{--} at high pH.

Table 2 shows that both in the presence and absence of micro-organisms, the total amount of phosphate taken up from all concentrations was greatest when the pH was low and that more was transferred to the shoots of plants supplied with the highest concentrations, irrespective of pH and microbial infection. However, although micro-organisms had a considerable influence on the transfer of phosphate to the shoots, except at the higher concentration this effect was much less pronounced at pH 4 than at pH 6 or 8. A similar effect of pH was apparent on the extent of microbial activity on the roots as indicated by the quantity of phosphate present as soluble esters and inorganic phosphate.

It is evident therefore that when micro-organisms are present, differences in the accumulation of phosphate at high and low pH do not necessarily reflect the functioning of separate carrier mechanisms for the two ion species (Hagen and Hopkins 1955).

3. *Effects of photosynthesis*

Since the microflora on and around the roots is dependent very largely for its supply of organic nutrients on exudation from the roots and breakdown of root tissues (Rovira 1965) factors affecting the synthetic processes of the plant are likely therefore to affect the metabolism of these organisms through their effect on exudation. For example, it has been shown by

TABLE 2

Effect of pH on the uptake and distribution of ^{32}P-labelled phosphate in young barley plants grown under sterile and non-sterile conditions. The plants were exposed to solutions of potassium phosphate of various concentrations at pH 4, 6 and 8 for 3 hours

pH	Conditions of plants	Concentration of phosphate ppm P			
		0·001	0·01	0·1	1·0
	Total uptake μgP/plant				
4	Sterile	0·40	3·12	24·68	95·7
	N. Sterile	0·156	2·81	28·06	100·6
6	Sterile	0·32	3·40	26·87	79·5
	N. Sterile	0·15	1·53	22·49	77·6
8	Sterile	0·23	0·94	11·09	53·8
	N. Sterile	0·13	1·34	14·61	64·2
	P transported to shoot as % total plant P				
4	Sterile	7·5	8·7	50·2	48·6
	N. Sterile	3·8	6·4	34·3	49·6
	Ratio Sterile/N. Sterile	2·0	1·3	1·5	1·0
6	Sterile	6·5	15·3	36·0	50·7
	N. Sterile	0·7	3·3	24·1	44·8
	Ratio Sterile/N. Sterile	9·3	4·6	1·5	1·1
8	Sterile	3·5	5·3	17·1	48·9
	N. Sterile	0·8	1·5	8·2	38·1
	Ratio Sterile/N. Sterile	4·4	3·5	2·1	1·3
	Soluble esters and inorganic P as % root P				
4	Sterile	81·1	82·5	86·3	87·7
	N. Sterile	43·5	65·3	62·8	75·2
	Ratio Sterile/N. Sterile	1·9	1·3	1·4	1·2
6	Sterile	80·0	78·9	81·2	88·0
	N. Sterile	37·2	46·6	58·3	71·3
	Ratio Sterile/N. Sterile	2·1	1·7	1·4	1·2
8	Sterile	76·5	82·6	82·3	85·2
	N. Sterile	29·3	22·3	52·3	70·6
	Ratio Sterile/N. Sterile	2·6	3·7	1·6	1·2

Rovira (1959) for tomato and clover plants in solution culture that a 60% decrease in light intensity caused considerable changes in the relative quantitites of different amino acids in the exudate and decreased the overall quantity exuded quite markedly. It is possible therefore that the accumulation of phosphate by micro-organisms on roots would be dependent to some extent on the carbohydrate status of the plant. Accordingly, the uptake and distribution of phosphate by 'starved' plants which had been grown for 24 hours in the dark was compared with that of 'normal' plants under sterile and non-sterile conditions. Uptake of phosphate was deter-

TABLE 3

Effect of carbohydrate status on the uptake and distribution of ^{32}P-labelled phosphate in young barley plants grown under sterile and non-sterile conditions. The plants were exposed to a solution of potassium phosphate containing 0·01 ppm P at pH 6 for 3 hours in the dark after 24 hours either in the light ('normal' plants) or in darkness ('starved' plants)

Carbohydrate status	Normal		Starved	
Condition of plants	Sterile	N. Sterile	Sterile	N. Sterile
Phosphate in roots				
μg P/plant	1·96	1·43	0·62	1·33
% in soluble esters/inorganic P	71·5	25·7	71·5	42·4
Phosphate in shoots				
μg P/plant	0·12	0·001	0·18	0·17
% total plant content	5·6	0·1	22·4	12·7
Phosphate removed from culture vessel walls with acidified carrier: μg P	0·087	0·377	0·065	0·187

mined from a solution of potassium phosphate containing 0·01 ppm P at pH 6 for 3 hours in the dark so that no photosynthate was produced during this period.

As in the previous experiments, micro-organisms caused a marked reduction in the fraction of the phosphate in roots which was present as soluble esters and inorganic P (Table 3). This effect was much greater in the 'normal' than in the 'starved' plants suggesting that there was less microbial activity in the latter. Some independent evidence of this was provided by the reduction in the quantity of phosphate which could be removed from the walls of the culture vessels with acidified carrier at the end of the experiment.

An interesting feature of the present results is that although under sterile conditions starvation reduced root content, it did not affect the fraction present in soluble esters; this suggests that the decrease in substrate limited uptake but had little effect on the partition of such phosphate as was absorbed between the soluble esters and nucleic acids. It is of interest also that in the absence of micro-organisms the actual quantity of phosphate transferred to the shoots was somewhat increased by starvation. In both these respects the uninfected plants contrasted markedly with those under non-sterile conditions thus providing further evidence that effects on the utilization of phosphate caused by metabolic factors in plants can be readily concealed by microbial activity.

DISCUSSION

As in any other aspect of plant nutrition it is difficult to extrapolate from the results of studies in solution culture to the possible situation of plants growing in soil. In the present work this is particularly so as apart from the different conditions for plant growth, micro-organisms in the soil are likely to be more numerous and diverse in their action. The very simple microflora in the present experiments associated with the plants grown under non-sterile conditions appeared merely to compete for nutrients. In contrast, the microflora of the rhizosphere may be expected to influence nutrition and the growth of plants in a variety of ways. The micro-organisms may act beneficially by transforming unavailable mineral and organic compounds in the soil into forms available for uptake by higher plants, by breaking down root secretions which have a toxic effect on plant growth, by producing substances which may accelerate plant growth and development and by producing antibiotics which may suppress the activities of parasitic organisms. Alternatively micro-organisms may act in a deleterious manner by competing with plants for available nutrients, by producing substances that may suppress growth and retard development and by parasitizing plants.

It is therefore of particular interest to compare results obtained in the present work with findings in studies in which soil micro-organisms have been used. In an investigation of the effects of a mixed suspension of soil micro-organisms on the absorption of phosphate by tomato and clover seedlings in solution culture Bowen and Rovira (1966) observed an increase in both uptake and translocation of phosphate, indicating possibly

that when a wide spectrum of organisms is present the stimulatory effects of microbial secretions may predominate over any competitive effect. In sand culture on the other hand Akhromeiko and Shestakova (1958) showed that the addition of rhizosphere organisms reduced the uptake by oak and ash seedlings of ^{32}P-labelled phosphate supplied as sodium dihydrogen phosphate. When, however, phosphate is supplied as insoluble compounds the opposite effect can sometimes be demonstrated. Gerretsen (1948) found that the addition of soil micro-organisms to previously sterile cultures caused insoluble phosphate containing compounds to be broken down with a resultant increase in the uptake of phosphorus by the plant. None the less, despite the widespread occurrence of organisms in the soil which can release phosphate from minerals when grown in isolation on agar plates, no marked increase in the uptake of phosphate by plants growing in soil has been demonstrated which can be ascribed to such microbial activity (Cooper 1959; Myśków 1961). Endophytic mycorrhiza alone have been unequivocally shown to increase uptake of phosphate from sparingly soluble compounds added to soil and then only for soils very low in native phosphate. (Daft and Nicolson 1966; Gerdemann 1964, 1965.)

All the above results, whether in sand, soil or water culture and showing increases or decreases in the uptake of phosphate by plants in response to the presence of micro-organisms have one factor in common, namely, that effects are only apparent when phosphate is in short supply. This appears to be the major criterion which determines the relative importance of microbial effects on the phosphate nutrition of higher plants. Given this condition, in any particular situation, other factors which can influence the composition and metabolic activity of the microflora, such as the pH and substrate availability through exudation, may well assume importance and induce differences in the growth and nutrition of the plants. This is a field of work which as yet has scarcely been tapped.

In extreme situations many problems regarding the inorganic nutrition of plants may be better understood if the microbiology of the situation were to be examined. For example, it is conceivable that the ability of *Mercurialis* to grow successfully in very shaded conditions, on soil very low in available phosphate (Pigott 1966) may in part reflect a very low production of exudates by the roots resulting in an impoverished and thus less competitive rhizosphere flora. However, such interpretations can at the moment be little more than speculation. None the less, it is evident that any aspect of plant nutrition will be inadequately understood unless account is taken of the activities of the countless and very diverse micro-organisms present.

ACKNOWLEDGEMENTS

I thank Dr R. Scott Russell for his stimulating interest in this work and Miss U.C. Frankenburg and Miss C. Lambourne for their valuable assistance.

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